

## Genetic Polymorphisms at Three Loci of PRLR and FSHR Gene Correlate with Litter Size in Chinese Haimen Goat

<sup>1</sup>Y.J. Li, <sup>1</sup>L. Zhang, <sup>1</sup>L.Q. Shang, <sup>1</sup>H.F. Wang, <sup>1</sup>H. Zou, <sup>2</sup>H. Zhang and <sup>1</sup>D.J. Ji  
<sup>1</sup>College of Animal Science and Technology, Yangzhou University, Yangzhou,  
Jiangsu Province 225009, P.R. China  
<sup>2</sup>Haimen Goats Breeding Farm of Government Management,  
Haimen, Jiangsu Province 226113, P.R. China

---

**Abstract:** Using PCR-SSCP method, the relationship between genetic polymorphisms at three reproduction associated loci and litter size were explored in Chinese Haimen goat. The results showed that intron 1 of Prolactin Receptor (PRLR) gene had two genotypes and three genotypes were also found in intron 2 of the same gene while no polymorphism was detected in FSHR gene. The least square analysis and multiple comparisons of the polymorphisms at PRLR gene loci and litter size indicated that variations of PRLR gene intron 2 correlated extremely significantly with litter size in Haimen goat. These findings demonstrated that PRLR gene could be used as a candidate genetic marker for fecundity in goat.

**Key words:** Haimen goat, litter size, PRLR, FSHR, PCR-SSCP, fecundity

---

### INTRODUCTION

Haimen goat is an exclusive goat species for high quality writing brush with wool and both its meat and skin performance in the world (Chen *et al.*, 2002). It has been listed on the national livestock and poultry breed resources protection directory of China. In recently years, some researches focus on its high fecundity showing that 25.0% of Haimen goats have 3 L, 19.6% have 4 L, 5.4% have 5 L and totally 50.0% have >3 L. When crossing with male Boer goat, 52.0% have >3 L.

As the heritability of litter size is so low (about 0.1), the effect of using traditional breeding method to improve this special advantage is limited. Molecular Marker-Assisted Selection (MAS) is now proved an effective breeding method. MAS can affect the time, the strength and the accuracy of the selection and can greatly improve the efficiency of the selection of a trait with a low heritability.

Prolactin (PRL) is an anterior pituitary peptide hormone with an important role in animal reproduction. Its function is regulated by the hormone's receptor (PRLR). The relationship between PRLR gene and reproductive performance of pigs (Rothschild *et al.*, 1998), cattle (Zhang *et al.*, 2007a), goats (Zhang *et al.*, 2007b), sheep (Zhu and Du, 2001) and other animals (Cassy *et al.*, 1998), have been recently studied. Because of its comprehensive interaction and effect in the process of reproduction, PRL gene is regarded as a candidate genetic marker for the trait

of propagation (Tiong *et al.*, 1992). Follicle-Stimulating Hormone Receptor (FSHR) can send signals of follicle stimulating growth and development in animal breeding activities and the link between FSHR gene polymorphism and reproductive traits like litter size is a hot topic in the research of animal genetics and breeding. The studies of cows (Gromoll *et al.*, 1996), sheep (Fry, 1998) and pigs (Linville *et al.*, 2001) have proved that FSHR gene polymorphisms affect the reproductive performance, it is also regarded as a candidate genetic marker for enhancing the litter size of animals.

In this study, PCR-SSCP method is used to explore the relationship between the polymorphism of these two genes and the litter size in Haimen goats for the purpose of developing and utilizing the genetic resources of multiple births and to establish polyembryonic breeding flock and new high fecundity strain of Haimen goats.

### MATERIALS AND METHODS

**Animals:** About 213 Haimen goats with breeding records were used in the study. All the goats were fed in the state-run Haimen goat breeding farm. Genomic DNA were extracted from ear tissue samples.

**PCR-SSCP detection:** Primers were designed according to the literature (Lei *et al.*, 2004; Jing, 2004) and synthesized by Shanghai Sangon Company. The primers are shown in Table 1.

**Table 1: The primers of the three loci**

Primer location	Amplicon length (bp)	Forward	Reverse
<b>M1</b>			
PRLR Intron1	215	CATCTGCTGGAGGTAAGTGC	TTCATTGCCCTCTGACGCTT
<b>M2</b>			
PRLR Intron2	176	TGTCAGTAAGCGTCAGAGGGC	GGCTGGTGAAGGTCACTCTT
<b>M3</b>			
FSHR Exon 10	230	ATCACGCTGGAAAGATGGCATAACC	GACATTGAGCACAAAGGAGGGAC

**A typical PCR reaction protocol was used:** About 4 min predenaturation at 95°C followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 61°C, 1 min extension at 72°C and a final extension of 10 min at 72°C. The PCR products were then detected on 2% agarose gels. Good PCR products were electrophoresized on 10% of the non-denaturing polyacrylamide gel at room temperature overnight and then stained with AgNO<sub>3</sub> method (Lu, 1993; Jing, 2001).

**Statistical analysis:** The following formula is adopted to process data:

$$\text{The degree of Locus homozygosis } J = \sum_{i=1}^n p_i^2$$

$$\text{The degree of Locus heterozygosis } h = 1 - \sum_{i=1}^n p_i^2$$

$$\text{The effective number of alleles } N_e = 1 / \sum_{i=1}^n p_i^2$$

In the formula,  $p_i$  ( $i = 1 \sim n$ ) means frequency of several alleles at a marked locus. Polymorphism Information Content (PIC):

$$PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2p_i p_j^2$$

In the formula of PIC,  $p_i$  and  $p_j$  are the frequency of different no. alleles in the group, respectively.  $N$  are the allele numbers. Hardy-Weinberg equilibrium testing on three gene loci:

$$\chi^2 = \frac{\sum_{i=1}^n (O_i - E_i)^2}{E_i}, df \geq 2$$

Where:

$O_i$  = Real value

$E_i$  = Theoretical value

$n$  = The number of allele

**The correlation analysis of genotype and litter size:** With the following model of least square analysis of variance, the correlation of litter size of Haimen goat and different genotypes in the three gene loci were analyzed:

$$y_{ijkl} = \mu + HYS_i + P_j + G_k + e_{ijkl}$$

Where:

$y_{ijkl}$  = The record of the litter size

$\mu$  = Population mean

$HYS_i$  = The effect of year and season in I-field

$P_j$  = The effect of No. j birthrank

$G_k$  = The effect of No. k marked genotype

$e_{ijkl}$  = The effect of random residual error. GLM of the SAS software (version 8.0) is used to complete the analysis process

## RESULTS AND DISCUSSION

**PCR-SSCP results at the three gene loci:** Intron1 of PRLR gene had two genotypes AA and AB and three genotypes CC, CD and DD were found in Intron 2 of PRLR gene. But the Exon 10 of FSHR gene had only one genotype HH (Fig. 1-3).

**Frequency of gene and genotype at the three loci:** We could found that BB-homozygous individuals were not detected in the study group and FSHR exon 10 has only one genotype (Table 2). Through Hardy-Weinberg equilibrium testing, the results of Chi-square testing showed that two loci of PRLR gene were in an extreme genetic imbalance state ( $t > t_{0.01}$ ), however FSHR gene exon 10 were homozygous.

**Genetic characteristics of 3 loci in Haimen goat:** We found that the allele number of the two loci of PRLR gene in Haimen goat were both two (Table 3). The heterozygosis degree, the effective allele numbers and the PIC in Intron 1 were all higher than those in Intron 2. These two loci had highly heterozygosis degree and they had moderate polymorphism ( $0.25 < PIC < 0.5$ ) as well. On the other hand, Exon 10 of FSHR had only one allele, this locus in Haimen goat was homozygotic.

**The correlation analysis between polymorphic locus genotypes and litter size:** As it can be shown from Table 4, different genotypes of polymorphic loci have different impacts on traits of the litter size. Two genotypes of PRLR intron 1 have no significant different impact on

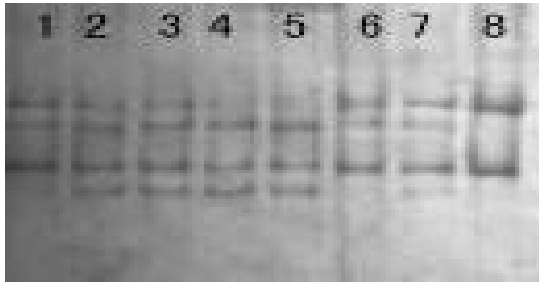


Fig. 1: Pattern of SSCP of PRLR gene Intron1: Lane 1-5, 7 were AB genotypes, lane 6 and 8 were AA genotypes



Fig. 2: Pattern of SSCP of PRLR gene Intron 2: Lane 3, 5, 8 were CC genotype; Lane 4, 6, 7 were CD genotype; Lane 1, 2 were DD genotype

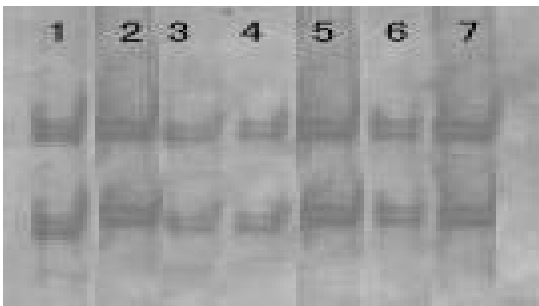


Fig. 3: Pattern of SSCP of FSHR gene Exon 10: Lane 1-7 were HH genotype

the trait of litter size ( $p>0.05$ ) on the other hand, three genotypes of PRLR intron 2 significantly influence trait of the litter size ( $p<0.01$ ) (Table 3). Two alleles of Intron 1 found in this study were homogeneous distribution. Gene frequency of A was 0.59 and B was 0.41. However, two alleles of Intron 2 were heterogeneous distribution, gene frequency of C was higher (0.79) than that of D (0.21). It is thought that these two loci were in the extreme genetic imbalance in Haimen goat and the majority was the superior genotype of lambing. The reason why BB

Table 2: Gene frequency and genotype frequency of three loci

Locus	Gene frequency		Genotype frequency		
	A	B	AA	AB	DD
PRLR	0.59	0.41	0.18	0.82	
Intron1					
PRLR	0.79	0.21	0.61	0.37	0.02
Intron2					
FSHR Exon 10	1		1		

Table 3: Genetic characteristics of 3 loci in Haimen goats

Locus	No. of alleles	J	h	No. E.A.	PIC
PRLR Intron1	2	0.5162	0.4838	1.9372	0.3668
PRLR Intron2	2	0.6682	0.3318	1.4966	0.2768
FSHR Exon 10	1	1	0	1	0

Table 4: Least squares mean and standard error for litter size of polymorphic loci of genotypes

Loci	Genotype	No. of samples	Least squares mean and standard error
PRLR Intron1	AA	38	2.35±0.27 <sup>a</sup>
	AB	175	2.47±0.09 <sup>a</sup>
PRLR Intron2	CC	130	2.57±0.14 <sup>A</sup>
	CD	78	2.12±0.20 <sup>B</sup>
	DD	5	2.05±0.53 <sup>B</sup>

The same letter means no significant difference,  $p>0.05$ , different capital letters means difference was extreme significant ( $p<0.01$ )

genotype was not found in Intron I was likely that the high-lambing individuals were obtained through artificial selection. Polymorphism of FSHR gene was not found in Haimen goat which was in accordance with Lan *et al.* (2006)'s reports indicating that the locus of FSHR was homozygosis or there had no mutation in this locus in Haimen goat. Average heterozygosis approximate reflects the mutation degree of genetic structure. The heterozygosity of the two loci of PRLR gene were 0.4838 and 0.3318, respectively indicating that these two loci had high heterozygosis and genetic diversity in Haimen goats and these two loci could be selected as candidate gene markers for breeding.

Otherwise, the heterozygosity of FSHR gene was zero indicating that this locus was homozygosis in Haimen goat and this locus was not suitable for a candidate gene marker. PIC is an ideal index to evaluate the polymorphism of gene fragment.  $PIC>0.5$ ,  $0.25<PIC<0.5$  and  $PIC<0.25$  mean that the locus has higher polymorphism, moderate polymorphism and lower polymorphism, respectively. The higher is PIC the greater heterozygote proportion a population has. The two loci of PRLR gene in this study were both moderate PIC site while the PIC of FSHR gene's locus was zero.

The two genotypes of PRLR intron 1 had no significant influence on litter size ( $p>0.05$ ) while the three genotypes of PRLR Intron 2 had extremely significant influence on litter size ( $p<0.01$ ). There might be two reasons for the relationship between the PRLR intron 2 and character of litter size. One is the minor multi-gene effect of PRLR gene on the litter size, the other is the

linkage between the loci of litter size trait and PRLR gene and in this gene cluster, PRLR gene was the main effect gene. Jing (2004) reported the correlation between PRLR gene polymorphism and goat litter size through research in Shandong Jining grey goat, Boer goat, Liaoning cashmere goat, Wendeng dairy goat and Beijing native goat. Kang (2006) also obtained the similar result in Heibei Jizhong goat. These studies all demonstrated that PRLR gene intron 2 had a significant influence on goat litter size. This study further proposed the relationship between PRLR gene and goat litter size and provided the effective means for breeding high fertility Haimen goats.

Much attention was paid on the FSHR gene after 1990s. Lan *et al.* (2006) found no polymorphism existing in FSHR gene Exon 10 in Xinong Sannon, Guanzhong goat, Shannan white goat, Angora goat and Boer goat groups. However, Ning (2006) found that FSHR gene exon 10 had polymorphism in Hebei Jizhong goat groups and the polymorphism related with the litter size. In this study, FSHR gene was selected as a candidate gene of high fertility in Haimen goat but no polymorphism was detected in FSHR gene exon 10 in Haimen goat which was in accordance with Lan *et al.* (2006)'s report.

### CONCLUSION

In this study, PRLR gene can be regarded as target candidate gene while FSHR gene was not suitable for the candidate gene of high fertility in Haimen goat. The mechanism of the functions of PRLR gene variations on breeding still need further research.

### ACKNOWLEDGEMENTS

This research was financially supported by the Agriculture New and High-tech project of Jiangsu Province, China (Grant NO:BG2006304).

### REFERENCES

- Cassy, S., M. Charlier, L. Belair, M. Guillomot, G. Charron, B. Bloch and J. Djiane, 1998. Developmental expression and localization of the prolactin receptor (PRLR) gene in ewe mammary gland during pregnancy and lactation: Estimation of the ratio of the two forms of PRLR messenger ribonucleic acid. *Biol. Reprod.*, 58: 1290-1296.
- Chen, Q.K., H.L. Qian and W.F. Sha, 2002. Reproductive performance and crossbreeding application of the Haimen goat. *J. Nanjing Agric. Technol. Coll.*, 18: 35-39.
- Fry, R.C., 1998. Induction of ovulation in chronically hypohysectomized Booroola ewes. *J. Reprod. Fertil.*, 82: 711-721.
- Gromoll, J., P. Elisabeth and E. Nieschlag, 1996. The structure and organization of the human Follicle-Stimulating Hormone Receptor (FSHR) gene. *Genomics*, 35: 308-311.
- Jing, D.Y., 2001. Guide of Fine Molecular Biology Experiments. Science Press, USA.
- Jing, Y., 2004. PCR-SSCP analysis on intron 1 and 2 of prolactin receptor gene in goats. Master Thesis, Shanxi Agricultural University, Taigu, Shangxi.
- Kang, F., 2006. Correlation on Single Nucleotide Polymorphisms (SNP) of prolactin receptor gene and prolificacy in goats. Master Thesis, Hebei Agricultural University, Baoding, Hebei.
- Lan, Y.X., H. Chen, C.Y. Chen, C.Y. Pan, C.Z. Lei, Y.D. Zhang and J. Yu, 2006. PCR-SSCP detection and DNA sequence analysis of exon 10 of goat Follicle-Stimulating Hormone Receptor (FSHR) gene. *J. Agric. Biotechnol.*, 14: 484-488.
- Lei, X., H. Chen, Z. Yuan and T. Xu, 2004. Single nucleotide polymorphism in cow follicle-stimulating hormone receptor gene and its association with bovine twinning traits. *Chinese Biochem. J.*, 20: 34-37.
- Linville, R.C., D. Pomp, R.K. Johnson and M.F. Rothschild, 2001. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. *J. Anim. Sci.*, 79: 60-67.
- Lu, S.D., 1993. Modern Techniques of Molecular Biology Experiments. Higher Education Press, Beijing, China.
- Ning, L.N., 2006. Characterization of partial region of the human Follicle-Stimulating Hormone Receptor (FSHR) gene. Master Thesis, Hebei Agricultural University, Baoding, Hebei.
- Rothschild, M.F., A.L. Vincent, C.K. Tuggle, G. Evans and T.H. Short *et al.*, 1998. A mutation in the prolactin receptor gene is associated with increased litter size in pigs. *Anim. Genet.*, 29: 60-74.
- Tiong, T.S., J.L. Stevenson and A.C. Herington, 1992. Regulation of prolactin receptor gene expression by thyroid hormone status in the rat. *J. Mol. Endocrinol.*, 18: 63-72.
- Zhang, G.X., M.X. Chu, J.Y. Wang, L. Fang and S.C. Ye, 2007a. Polymorphism of exon 10 of prolactin receptor gene and its relationship with prolificacy of Jining grey goats. *Hereditas*, 29: 329-336.
- Zhang, G.L., L.S. Zan and L.G. Shen, 2007b. Association between hinf polymorphism of prolactin receptor (PRLR) gene and performance of dairy cows. *Chinese J. Anim. Vet. Sci.*, 38: 888-892.
- Zhu, J. and L.X. Du, 2001. The progress of candidate genes for fecundity in small tail han sheep. *Anim. Sci. Vet. Med.*, 24: 31-33.